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TWO CONIDIA-BEARING FUNGI.[1]

CUNNINGHAMELLA AND THAMNOCEPHALIS, N. GEN.

A. F. BLAKESLEE.

(WITH PLATE VI)

CUNNINGHAMELLA ECHINULATA Thaxter.

Oedocephalum echinulatum Thaxter, BOT. GAZETTE **16**: 17. *pl. 4. figs. 8–11.* 1891; Saccardo, Sylloge Fungorum **10**:522; Lindau, Engler-Prantl's Pflanzenfamilien **1**[1]:426. *figs. 220 A-B.*

Cunninghamella africana Matruchot, Annales Mycologici **1**:45–60. *pl. 1.* 1903.

Cunninghamella echinulata Thaxter, Rhodora **5**:97. 1903.

"Une Mucorinée purement conidienne, *Cunninghamella africana*" is the title under which MATRUCHOT (*l. c.*) has described an Oedocephalum-like fungus which on morphological and physiological grounds he considered was to be included among the Mucorineae. The species had been previously described by THAXTER, however, as *Oedocephalum echinulatum*, and with this name has been included among the Oedocephalums in SACCARDO'S *Sylloge*, where the similarity to Choanephora is pointed out.

The mucors are typically coenocytes with a non-septate mycelium at least in the early stages of development, with sexually formed zygospores, and with the characteristic production of endogenous non-sexual spores within sporangia. The absence of septa in the hyphae of the species under consideration led MATRUCHOT, despite the lack of sporangia or zygospores, to believe that he might be dealing with a member of the Mucorineae. Piptocephalis, which is an

[1] This paper was written while working under a grant from the Carnegie Institution.

obligate parasite upon various mucors, he found would grow on Cunninghamella as host, but on none of a considerable number of non-mucor forms from representative groups of the higher and lower fungi. From the results of this ingenious test of parasitism, and from the vegetative structure, he decided that Cunninghamella was to be placed in a distinct group of the Mucorineae alongside of Choanephora, where Oedocephalum-like fructifications occur in addition to sporangia. The discovery of the zygospores of this species by the writer[2] has established beyond question its position among the Mucorineae, and renders not improbable that a further cultural investigation may similarly give independence to others of the *Fungi imperfecti*. Since the method of finding the sexual form of reproduction in this species is that which recently the writer has adopted in obtaining, among others, the zygospores of *Syncephalastrum*, *Absidia repens*, *Helicostylum*, and *Circinella umbellata*, for which, with the exception of the last species, zygospores had never been known, it seems not inappropriate to give a brief account of their discovery in Cunninghamella. It is believed, moreover, that an application of similar methods may lead to a clearing up of some of the present anomalies in fruit production encountered in other of the fungi as well as in the algae.

According to their method of sexual reproduction, the Mucorineae have been divided into two main groups, homothallic and heterothallic, characterized respectively by bisexual and unisexual thalli.[3] In the forms known to belong to the homothallic group, zygospores are produced along with the non-sexual sporangial spores under normal cultural conditions, and for this reason the majority of them have been kept under cultivation with a constant production of zygospores for many years. No new members have been added to this group during the present year's investigation, while the accession of a number of forms to the heterothallic group further emphasizes the conclusion that this latter group comprises a very large majority of the species. The (+) and (−) sexual strains of heterothallic forms were first obtained analytically from those few fortunate cultures in

[2] Sexual reproduction in the Mucorineae. Proc. Am. Acad. **40**:311. 1904.

[3] *Loc. cit.* and Zygospore formation a sexual process. Science N. S. **19**:864–866. 1904.

which it had been shown, by the production of their zygospores, that the two opposite strains were present together. By a sufficient accumulation of material from different sources one may expect eventually to obtain the two sexual strains, and by their synthesis the zygospores as well of those forms in which the sexes are separate on different mycelia.

Cunninghamella, although reported by only two previous investigators, is not extremely rare. ATKINSON writes that he has had the species in cultures several times, and it has appeared occasionally in the Harvard laboratory, especially on material from the tropics. A pure culture of the fungus was thus obtained from dried flowers collected by the writer in Venezuela, and when contrasted in a culture between the (+) and (−) strains of a test species was shown to be (+) in character by the formation of imperfect hybrids with the (−) strain tested (*l. c. pl. 2. figs. 36-39*). Later a culture was secured from Porto Rico, and upon being similarly tested proved to be (−). The (+) and (−) strains thus determined were at once mutually contrasted and, as was to be expected, yielded an abundant production of zygospores. These cultures were made at laboratory temperature during warm weather in the latter part of July. In the fall the experiment was repeated; but although the two (+) and (−) strains separately continued to produce imperfect hybrids with (−) and (+) test strains respectively, yet when under the same cultural conditions contrasts were made between the two strains themselves no zygospores resulted. A series of cultures under various external conditions demonstrated that with this species the temperature is one of the most important factors to be considered in securing a formation of zygospores. At 20° C. zygospores have not been obtained; but at temperatures from 25° to 34° inclusive, zygospores readily form on the usual culture media employed in the laboratory. It is certainly remarkable that under any conditions the sexual response should be less intense when the (+) and (−) strains of a given species are contrasted together than when they are contrasted against strains of a different species, but Cunninghamella is not unique in this respect. A like condition has already been noted in a species of the genus Mucor (*l. c.* 308, diagram), and a number of other forms more recently investigated show a similar behavior.

Figs. 1–5 were taken from test tube material and illustrate stages in conjugation. The progametes, as also the gametes, are frequently unequal, but this fact, as in the case of Rhizopus (*l. c.* 269), is probably associated merely with an inequality in the amount of nutriment received from the opposite zygophoric hyphae and has no sexual significance. The zygospores vary in size from $46 \times 40\mu$ to $80 \times 63\mu$, and average about $70 \times 58\mu$, with the longest diameter at right angles to the suspensors. The mature zygospore (*fig. 4*) is nearly opaque and thickly beset with comparatively long spines, which frequently, however, seem to be more or less arrested in their development, so that individual zygospores taken from the same culture may present a considerable difference in appearance. In Van Tieghem cells, where the amount of nutrient is necessarily scant, conjugation has not been directly followed under the microscope. In test tube cultures zygospores form chiefly in the lower parts of the tube below the conidial fructifications, producing a reddish-brown mass of minute specks which singly are hardly noticeable without the aid of a hand lens. Hyphae from which progametes are developed do not as a rule take part in conidial formation, yet by a careful search one may find instances showing the two forms of fructification in direct connection with the same hypha (*fig. 3*). Whether or not the zygophoric hyphae are mutually attractive, as is the case with many forms, has not been determined. The contact, however, of sexually opposite hyphae seems to be a stimulus to an increased branching, for in the region of zygospore formation the conjugative hyphae are much branched and closely entangled. A scalariform arrangement of the zygospores is common, and progametes may occasionally form so close together on two adjacent filaments as to give rise to twin zygospores (*fig. 5*) supported apparently by forked suspensors. Instances have also been observed in which one side alone shows an apparent forking of the suspensor, and in other rare cases the suspensor of one zygospore has the appearance of a side branch from that of another. The usual condition is that figured (*figs. 1–4*), where the progametes are developed laterally from adjacent hyphae at their points of contact. One zygophoric hypha may be laterally met by the termination of the other, but it is certainly seldom the case that their contact is exactly terminal.

In many heterothallic species the (−) is distinguished from the (+) strain by any one of a number of different characters, which in general indicate a less luxuriance in vegetative growth. No characters have been found as yet, however, by which one can distinguish between the sexual strains of Cunninghamella when grown apart in pure cultures.

Sufficient material has not been investigated to enable one to determine the relative abundance in nature of the strains of this species. In addition to the two (+) and (−) strains from Venezuela and Porto Rico already mentioned, a culture originally obtained from horse dung has been kindly communicated to the writer by ATKINSON, and the same species has recently been found on a specimen of dung kindly sent from the Philippines by COPELAND. Of these two latter, one is (−) and the other (+), so that in the four cultures tested none are neutral, and the (+) and (−) strains are equally represented.

THAMNOCEPHALIS, n. gen.

Vegetative hyphae fine, continuous, anastomosing. Fructifications erect, consisting of a main stalk supported above the substratum by stout rhizoidal props and bearing a bushy crown of subdichotomously branched fertile hyphae terminated by sterile branches. Spores solitary, borne on the surface of spherical heads. Heads borne at the apex of short lateral stalks which arise at nodes from opposite sides of the fertile hyphae at right angles to their planes of branching.

Thamnocephalis quadrupedata, n. sp.

Vegetative hyphae delicate, about 3μ in diameter, branched and freely anastomosing. Fructifications scattered, rose-brown, tree-like, about 0.75^{mm} tall. Main stalk thick-walled, tapering from about 15μ at base to about 8μ wide at apex, supported at maturity by two pairs of stout rhizoidal props which are anchored to the substratum by branches given off from their lower ends. The shriveled remnant of a fifth rhizoid hangs down midway between the two pairs of props, and a beak-like projection occurs on the side opposite the main stalk as the remains of an abortive secondary erect stalk. Hyphae of the crown 7–10 times dichotomously or subdichotomously branched, the planes of dichotomy being successively at right angles

to one another. At the first 6–8 nodes are laterally produced short conical or barrel-shaped branches, generally septate at base and septate terminally at junction with sporiferous head. Heads spherical, from about 19μ in diameter at first node to about 13μ in diameter toward the periphery, produced in acropetal succession and bearing the spores on slight papillae. Spores spherical, about 5.5μ in diameter, yellowish, thick-walled, very finely echinulate, ripening on the different heads in acropetal succession. Ultimate branches curved, sterile, often beset with protuberances on their convex sides, becoming septate, shriveled, and frequently abstricted before spore maturation. Hyphae of rhizoids, stalk, and crown becoming septate about the time of spore maturation.

Growing in a gross dung culture on fresh sphagnum, Cambridge, Mass.

This fungus appeared in the Harvard laboratory in the fall of 1902. An undetermined sample of dung had been placed in a crystallizing dish with fresh unsterilized sphagnum, and yielded in the course of time a scanty growth of mucors. Somewhat later the peculiar fructifications of this species were found already covering the layer of filter paper used in the culture like a grove of microscopic trees, and extending into the loosely packed sphagnum below. A large variety of substrata was tried in the attempt to obtain a pure culture of the fungus; and mass transfers from the original culture, which for several days continued to produce new fructifications, were made to dung and to fresh sphagnum, but it seemed impossible to elicit a growth in new cultures.

A few spore germinations were obtained in Van Tieghem cells on horse dung agar. The spores that germinated did so in two days, swelling to about 8μ in diameter before emitting slender germ tubes, simple or slightly branched. The longest germ tube observed reached but 170μ and no further growth could be obtained. The action of the fungus in the cultures attempted suggested that the species might have been parasitic upon the scanty growth of mucor which had developed in the original culture, and though it did not seem possible to stimulate growth by an admixture of this same mucor, the frequent irregular behavior of Syncephalis under similar circumstances does not render such a condition entirely improbable.

The origin of the fungus in the culture where it was found is uncertain. It is possible that it was introduced along with the fresh sphagnum, since its growth had no apparent connection with the dung. Fresh sphagnum was accordingly gathered at a later date from the same locality as before and used in unsterilized cultures, but failed to give further rise to Thamnocephalis. The improbability of finding the form again reconciles the writer to attempting to patch together the history of development from the somewhat scanty material at his disposal, which is especially lacking in young stages. It is believed, however, that in the main the process can be followed.

Anastomoses are common between overlying hyphae (*fig. 6*), and it is apparently at these places of anastomosing that the rudiments of the fructifications are formed. The condition shown in *fig. 7* has several representatives in the preparations saved, but the stages between it and that shown in *fig. 8* are entirely lacking. A comparison of the fructification as seen in *fig. 8* with a caricature of some giraffe-like creature will furnish terms convenient for discussion. Upwards of a hundred specimens near maturity have been examined and no deviations from the main type of fructification have been observed. A short narrow body supported by fore and hind legs bears on the dorsal side anteriorly a long neck supporting the fertile head of branches and posteriorly a short erect tail; while an umbilical cord connecting ventrally with the substratum probably serves as the chief channel for the influx of nutriment. It seems probable that in *fig. 7* we have represented the umbilical cord in the process of giving rise by branching to the short body which in turn is further dividing. Under this assumption the first branching of the stalk arising from an anastomosis would give rise to the body, the second to the neck and tail and to the short extensions of the body, from which by a further branching the two pairs of legs are developed. The tail becomes septate and shrivels at a very early stage, and in none of the material at hand has it been found entirely filled with protoplasm. In the specimen figured (*fig. 8*), however, and in one other the walls seem to be continuous around the end. This being the case, the tail arises from a branching of the same order as the neck, but becomes functionless; while the latter, perhaps because of

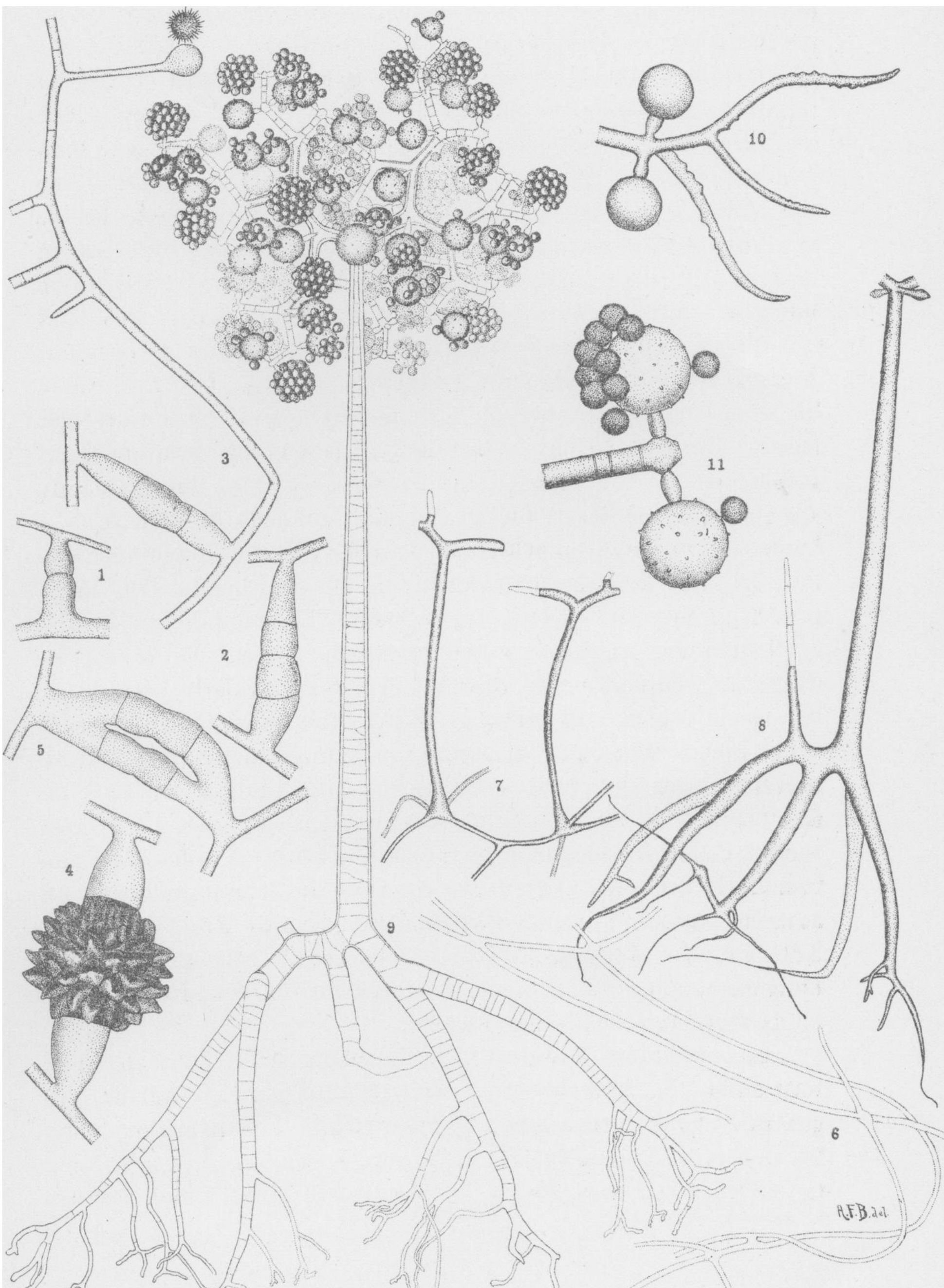
priority in origin, takes the ascendancy and appropriates the protoplasm for the development of the sporiferous head.

That the umbilical stalk is formed directly from the stem arising from an anastomosis and is at least the chief channel through which the fructification receives its supply of nourishment is indicated by a number of facts. Its base is at first in connection with the fine anastomosing hyphae, but as the plant reaches its full size it shrivels and usually leaves at maturity little more of a remnant than shown in *fig. 9*. In cases in which its base remains, it may be seen to be somewhat raised above the attachment of the two pairs of legs, as if it had been torn out of the substratum by the growth of these latter. The legs are obviously a later production, as is seen by *fig. 8*, where the anchoring rhizoids are just beginning to be given off toward their bases. The legs, as may be seen in the left hind leg of the specimen just mentioned, are supplied with rhizoids which at times certainly are comparatively short and end blindly; although in other cases a few of the rhizoidal branches can be followed for a certain distance into delicate filaments characteristic of the mycelium. This latter condition, however, is not so typical as for the umbilical stalk.

Neither the vegetative mycelium nor the hyphae of the fructification are septate during growth. Septation regularly takes place in the neck, legs, and branches of the head when the protoplasm which they contain becomes used up in spore-formation, and mature spores showing the characteristic echinulations are to be found on fructifications in which no septa have as yet been found. The cross walls are thin in comparison with the thick lateral walls and quite irregularly disposed. Often one connects the lateral wall with an adjacent septum or extends as a shelf but part way across the hypha. The branches of the crown easily separate at their septa, and by judicious tapping with a needle the crown may be denuded of nearly all its sporiferous heads and branches.

The only known form to which Thamnocephalis shows any close relationship is *Sigmoidiomyces dispiroides* Thaxter,[4] and the two genera evidently form a group by themselves. The method of spore-

[4] THAXTER, R., North American Hyphomycetes. BOT. GAZETTE **16**:22. *pl. 4. figs. 15–18.* 1891. Figures reproduced in ENGLER and PRANTL'S Pflanzenfamilien 1[1]:427. *figs. 220 G–H.*



BLAKESLEE on CUNNINGHAMELLA and THAMNOCEPHALIS

formation on paired heads which are located at points of branching of the fertile hyphae is essentially the same for both species. The outgrowths on the convexities of the sterile branches in Sigmoidiomyces are represented in Thamnocephalis by mere protuberances; the spores and their echinulations are much reduced in the latter; and the branches of the fertile hyphae are not markedly curved in the mature condition. Sigmoideomyces fails, moreover, to show that striking differentiation into fertile crown, main stalk, and rhizoidal props, which is a unique feature of Thamnocephalis. Sigmoideomyces was described from mature material, and it was not possible to determine the nature of the mycelium nor the time of septation of the fertile hyphae, but the similarity in the two forms would suggest that much the same condition exists as in Thamnocephalis.

MATRUCHOT has emphasized the systematic importance of nonseptate hyphae, and the condition in Cunninghamella shows that the presence of sporangia is not a *sine qua non* for admission to the company of the mucors. The absence of septa in young growing hyphae is a mucor character, but hardly less characteristic is their presence in varying abundance in older hyphae from which the protoplasm has been withdrawn. Cunninghamella is no exception to this statement (*fig. 3*), but perhaps Piptocephalis and *Spinellus macrocarpus* furnish as striking examples of septation in fertile hyphae as the group affords. The delicate anastomosing mycelial hyphae further remind one of the similar condition in Syncephalis. THAXTER (*l. c.*), MATRUCHOT (*l. c.*), and others have recognized the possibility of Rhopalomyces belonging to the Mucorineae. Without evidence from cultures the relationship of Sigmoideomyces and Thamnocephalis with the mucors must be left as only a suggestion.

Microscopic preparations and herbarium material of *Thamnocephalis quadrupedata* have been deposited in the Cryptogamic Herbarium of Harvard University.

NAPLES BIOLOGICAL STATION.

EXPLANATION OF PLATE VI.

The drawings were outlined with the aid of a camera lucida with the combination of Leitz and Bausch & Lomb lenses noted and have been reduced about one-quarter in reproduction.

Cunninghamella echinulata Thaxter.

FIG. 1. Development of progametes; obj. 7. oc. 3.

FIG. 2. Abstriction of gametes; obj. 7. oc. 3.

FIG. 3. Young zygote in connection with short conidiophore through hypha which has lost its protoplasm and become septate; obj. 7. oc. 3.

FIG. 4. Mature zygospore; obj. 7. oc. 3.

FIG. 5. "Twinned" zygotes; obj. 7. oc. 3.

Thamnocephalis quadrupedata, n. sp.

FIG. 6. Hyphae of mycelium showing anastomoses; obj. 7. oc. 3.

FIG. 7. Early stages of development of fructification; obj. 7. oc. 1.

FIG. 8. Young fructification showing basal portion and formation of sporiferous heads at first node of the crown; obj. 7. oc. 1.

FIG. 9. Mature fructification from which many of the fertile branches and spores have been shed; obj. D. oc. 2.

FIG. 10. Last fertile node of crown showing sterile terminal branches and young sporiferous heads from which the spores have not yet developed; obj. $\frac{1}{12}$. oc. 3.

FIG. 11. Fifth fertile node with sporiferous heads and spores; obj. $\frac{1}{12}$. oc. 3.